Encapsulation of Orange oil using fluidized bed granulation

A Thesis
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Adviser: Dr. Gary Reineccius

MAY 2019

Acknowledgements

Many personalities have shaped my path towards obtaining a master's degree in food science. Firstly, I would like to express my sincere gratitude to my advisor Dr. Gary Reineccius for giving me an opportunity to work in flavor research laboratory under his esteemed guidance. Throughout my research process, he has helped me to grow as an independent researcher. I am sincerely thankful to him for his patience and motivation.

I am also very thankful towards Dr. Tonya Schoenfuss and Dr. Mrinal Bhattacharya for accepting my request to serve on my exam committee.

I extend my special thanks to the Department of Food science and nutrition at University of Minnesota for giving me an opportunity to finish M.S degree in the department. Also, I would like to appreciate the presence of my classmates, lab mates and friends that I have made during this journey. Last but not the least, I would like to thank my parents for supporting me in all respect to complete my studies abroad.

Dedication

This thesis is dedicated to my devoted parents who have been my source of strength throughout this process. Their passion and hard work to follow their dreams has always inspired me. Thanks a lot for being the support system to me.

Abstract

The objective of this research was to determine if orange oil can be spray dried and agglomerated in a single step process called granulation and if so, to compare the products prepared using granulation to the traditional processes of spray drying and then agglomerating. The processes that were studied as a part of this research were spray drying (SD - A conventional process widely used in industry), agglomeration of spray dried Flavors (SDA) and fluidized bed granulation (FBG).

The model system used to evaluate the two different processes was an orange oil emulsion. They were made by first preparing a slurry 55% in solids (carrier in water). The carrier was a blend of Maltodextrin (MD-150 as bulking agent) and Modified Starch (Capsul™, an Octenylsuccinate acid derivatized starch as emulsifier). Orange oil was added at 25% of the carrier solids level and emulsion was made using a high shear rotor-stator blender. The said emulsion was subjected to spray drying and the resultant spray dried emulsion was agglomerated. The emulsion with same composition was also used for fluidized bed granulation (FBG). The resultant product, orange oil encapsulates were analyzed for Limonene Oxide content under storage (Shelf life study), Particle Size, Density, Orange Oil retention and Moisture Content.

It was observed that FBG samples had higher density than SD and SDA samples. Density was found to be proportional to run time of the process and SDA samples had higher density than SD samples.

Under the study operating conditions, fluidized bed granulation produced larger particles compared to spray drying or SDA. As one would expect particle size was found to be proportional to run time of the process (FBG and SDA). In case of FBG, it can be explained by the continuous spraying of emulsion while processing, which results continuous drying and film formation on granules (particle growth).

Under the study operating conditions, FBG products also had a lower moisture content compared to other products. FBG samples had the highest oil retention followed by spray drying and SDA; The SDA batch with shortest run time (about 30 mins) had the least oil retention.

While conducting the shelf life study, it was observed that granulation samples had the lowest limonene oxide content, hence less oxidation after four weeks of storage followed by agglomerated samples. Spray dried samples were observed to have highest limonene oxide content and hence, the highest oxidation after four weeks of storage as smaller particle size contributes to a higher available surface area per unit volume for oxidation.

Overall, it was observed during this study that fluidized bed granulation produced orange oil encapsulates that possessed better properties such as more resistance to oxidation, better retention of flavors and higher density than spray dried orange oil.

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Chapter I: Literature Review

A. Introduction

Food flavors are a primary determinant of the overall acceptability of a food product and therefore they are an important sensory attribute. Hence, flavor stability plays an important role in determining the quality of a food product. However, flavor loss from the food products which takes place during handling and storage of the food products decreases the acceptability. The flavor loss may take place due to evaporation, degradation, reaction of flavor compounds with other component of the food product or with the packaging material and oxidation due to exposure to air, heat and light. As food flavor is paramount for the preservation of food quality, a lot of research has been carried out in recent decades regarding flavor protection and preservation.

Encapsulation has been an effective way to maintain and protect flavor compounds. Microencapsulation provides protection against evaporation and avoids unwanted reactions during storage. (Wang et al., 2014). Therefore, the use of encapsulation for increasing the shelf life of manufactured products is widespread in pharmaceutical, food, paper and cosmetic industries. (Risch and Reineccius, 1995).

Microencapsulation can be defined as a process in which particles are coated or enveloped by a non-reactive solid material known as shell, wall, external phase or coating (Gouin, 2014). The material which is to be encapsulated is known by several terminologies such as core, internal phase, payload or active material.

Reasons for encapsulation

As mentioned above, encapsulation is used to maintain stability and integrity of flavor compounds. It can limit loss of flavor during storage.

Encapsulation may be performed for reasons such as

1) To protect the core/active material from environment.
2) To prevent undesirable reactions between flavors and active food ingredients.
3) To control the release of active ingredient as desired.

4) Ease of handling during transportation and usage due to conversion of liquid flavors into solid state, powdered encapsulates.

A wide variety of methods are used in industry for flavor encapsulation such as spray drying, spray chilling, coacervation, liposome entrapment and fluidized coating. (Gouin, 2014). According to Reineccius (2004), spray drying was the initial process which was used for flavor encapsulation and is still widespread and most common process used in flavor industries due to availability and low cost of spray drying process, ease of handling of the spray dryer, high retention of volatile components in the product and good shelf life properties.

The processes that were studied as a part of this research were:

Spray drying (SD - A conventional process widely used in industry),

Agglomeration of spray dried Flavors (SDA)

Fluidized bed granulation (FBG).

A wide variety of ingredients can be used as carrier materials for encapsulating flavor compounds. Very detailed research has been carried out in the field of flavor technology to identify the materials most suitable to be used as encapsulation carrier materials.

Important parameters that an excellent carrier material should comply to are:

1) Good emulsifying and film-forming properties.
2) Should be tasteless so as not to interfere in the taste of the product.
3) Should not react with core material and should be inexpensive.
4) Should exhibit low hygroscopicity and low viscosity in solution.

Three most common classes of materials used of encapsulating flavors are – **Gums (e.g. -Gum Acacia)**, **Starch Hydrolysates (Maltodextrins and corn syrup solids)** and **chemically modified starch such as (OSaN) Octenyl succinic acid derivatized starch).**

Maltodextrins are starch derivatives (acid or enzyme catalyzed starch hydrolysates) whose Dextrose equivalent (DE) value is less than 20. They are made using treatment of starch using acids or enzymes. (Reineccius, 2009). Maltodextrins are creamy white

hygroscopic polysaccharide powders which are almost tasteless. They contribute to the emulsion viscosity but offer no emulsification properties. Hence, they are commonly used in blends alongside either Gum Acacia or Modified starches. Octenyl succinic acid derivatized starch was chosen over other starch derivatives such as cyclodextrins and hydrolyzed starches for their excellent emulsification properties and higher encapsulation efficiencies for flavor compounds. Higher encapsulation efficiency results in lower surface oil content after encapsulation which contributes towards higher process efficiency and lower oxidation as surface oil is easily oxidizable. (Wang and others, 2014). Higher DEs, offer better protection against oxidation. Although higher DE maltodextrins are difficult to dry and offer poor flavor retention. Inversely, modified starches offer good encapsulation efficiency but offer poor protection against oxidation during storage time. Hence, blends of Maltodextrin + Modified starch are preferred to cover up these shortcomings of individual carrier solids.

It is important that the choice of carrier material be carefully considered as it greatly influences flavor retention during the encapsulation processes and oxidation during subsequent storage until consumption (Buffo et al., 2002). The choice of carrier affects many of the encapsulated powder characteristics including: particle size and shape, absolute and bulk density, flowability, dispersibility, moisture content, appearance, flavor load, shelf-life, stability to caking, structural strength, and release properties (Reineccius, 2004). There is not one single wall material that exhibits all the properties of a perfect carrier. Because of this, blends of different carrier materials are used to improve encapsulation properties.

Encapsulated products can possess following possible benefits (Zuidam and Shimoni, 2010):

1. Superior handling of core material (Conversion of liquid flavor into a powdered form which can be free flowing and easy to transport).
2. Immobility of active agent/ core material in food systems
3. Improved safety such as decreased flammability of volatiles like aroma.
4. Improved stability during processing and in final product due to less evaporation of volatiles and protection from oxidation.

5. Adjustable properties such as particle size, structure, solubility and color.
6. Off taste masking.

B. Spray Drying

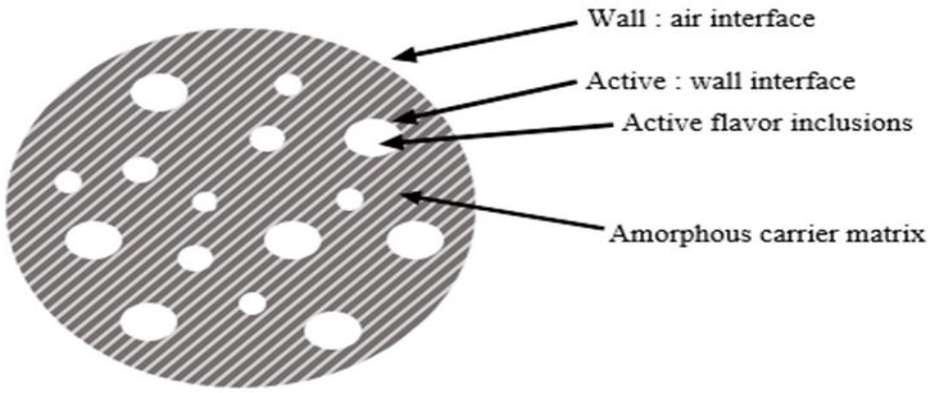

Figure 1. Illustration of a spray dried encapsulated powder particle figure (Reineccius and Yan, 2016).

Spray drying is the most widely used encapsulation method by flavor industries. It is used to produce about 90% of the encapsulated flavorings in the market. (Reineccius, 2009). It can be defined as transformation of fluid feed material to a dried particulate form by spraying the feed into hot air. Spray drying is performed on flavorings in order to capture volatile components, decrease the rate of oxidation, improve the handling properties by converting fluid material into powdered form and to control flavor release into final product. (Anantharamkrishnan and Reineccius, 2017).

For encapsulating a flavor compound using spray drying, an O/W emulsion is prepared. The carrier solid material is first dissolved in water and after the carrier solid is completely dissolved in water, flavoring (such as orange oil) is added. The emulsion is pumped into the drying chamber and is atomized (pressure spray, two fluid spray or centrifugal wheel atomization) into a stream of hot air, which rapidly dries the microencapsulates trapping the flavor inside the dry particles and this results in the formation of encapsulates in powdered form. (Risch and Reineccius, 1988). During spray drying, a film is formed at the droplet surface, thereby retarding the movement of larger flavor molecules, while smaller molecules are evaporated. Thus, flavor

molecules are selectively sealed under the film formed by carrier solids. The product is collected via cyclone collectors. During this process, flavor particle temperature never exceeds exit air temperature. (Reineccius, 2009).

The flavor emulsion being used for spray drying process should have a high solids content and low viscosity as possible to allow pumping and emulsion should not break down until it is atomized in spray dryer as emulsion breakage might cause explosions due to combustion of volatile compounds(flavorings) inside spray dryers. (Reineccius, 2009). But higher solids content means higher viscosity, which might slow down the film formation at droplet surface and hence promote the loss of flavor molecules. (Zuidam and Heinrich,2010). The encapsulation matrix should not become hygroscopic at high temperatures to allow efficient drying and should protect flavoring from degradation and evaporation during storage.

As shown in Figure 1, matrix or multiple core type of encapsulates are formed after spray drying.

The advantages of the spray drying as listed by Zuidam and Heinrich (2010) are:

1. Continuous production – Product can be collected at collection chamber continuously.
2. A well-known and widely used method with a lot of literature and both industry and research experience.
3. Wide variety of carrier materials can be used.

Some disadvantages of spray drying include:

1. Volatile flavors can be lost during spray drying. (Reineccius,2004)
2. Oxidation of heat sensitive flavors during spray drying.
3. Spray dried powders have small particle size and low density which causes poor handling properties and dusting problems.
4. They also have low dispersibility in water.

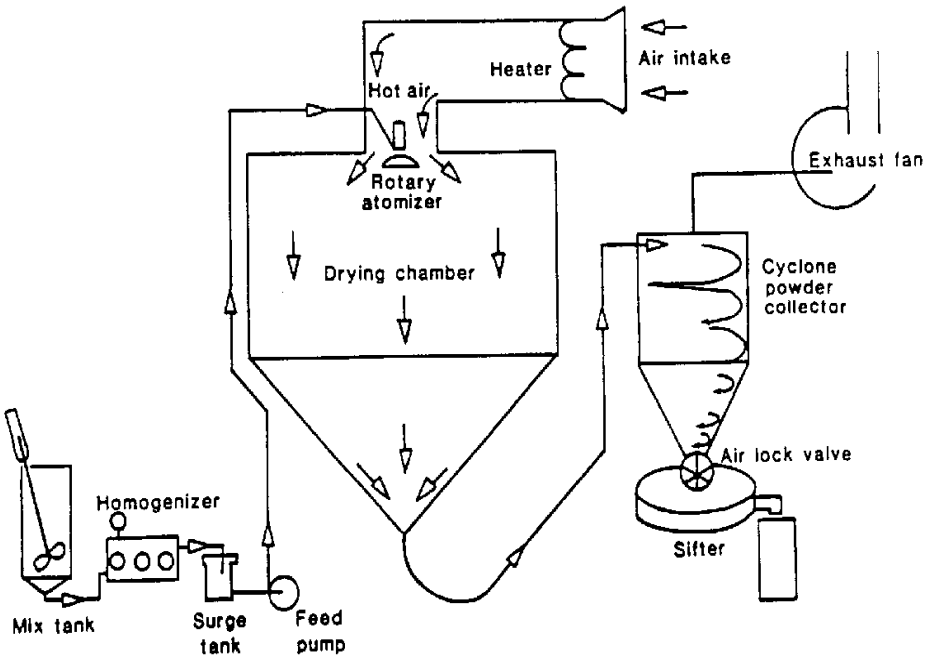

Figure 2. Typical spray drying assembly. Adapted from Reineccius, (2009).

C. Agglomeration

As mentioned in previous section, spray dried powders have small particle size and low density which causes poor handling properties and dusting problems. They also have low dispersibility in water. So, agglomeration is performed on spray dried powders to improve the particle size and dispersibility. Increase in flowability and wettability are also observed. An increase in particle diameter up to 150 μm has been reported in the literature. (Fuchs et al., 2006).

Agglomeration can be defined as a unit operation during which particles are assembled to form larger particles, called agglomerates. (Cuq et al.,2013) According to Buffo et al (2001), Agglomeration is a unit operation during which particle assembly takes place which results in formation of particles with larger size. To agglomerate particles, it is required that they are kept in contact with each other for some time which is usually achieved by outside forces.

The type of agglomeration used in this study was fluidized bed agglomeration, a type of wet growth agglomeration. As opposed to dry agglomeration, where particles are kept into contact using external pressure (also known as pressure agglomeration or compaction), wet growth agglomeration involves use of a binder liquid to establish particle contact. That contact is established between particles by moving them close to each other and cohesive forces are applied through wetting, which results in formation of liquid bridges between particles, which cause adhesion. After the wetting step, particles are subjected to drying, which results in formation of solid bridges between particles. Particles are subjected to alternate wetting and drying until desired particle size is obtained.

A fluidized bed is formed by passing hot air through the spray dried flavorings kept at the bottom of agglomerator and hence, causing movement of particles inside the chamber to form a fluidized bed; where the spray dried flavoring acts like a fluid. This type of fluidization is termed as gas-solid fluidization. Fluidized bed allows for efficient transfer of energy and a large amount of product can be handled during operations. These processes can be run in both batch and continuous mode.

Wet growth agglomeration is considered as the most suitable methodology for agglomeration of spray dried flavors as wall materials used in this method can easily dissolve in water and form strong interparticle bridges on re-drying. This is done by introducing a liquid binder, either in the form of condensing vapor, as liquid spray, or a mixture of both. (Schubert,1993). In this research, water was used as a binder and liquid spray was used as a method for binder delivery.

A batch system was used in this research due to availability constraints. Batch systems, when compared to continuous systems, offer limited throughput and higher operating costs (Risch and Reineccius, 1988).

Figure 3. Diagram of bottom spray fluidized agglomerator (courtesy of Glatt Air Techniques, Inc, Ramsey, NJ).

D. Fluidized bed granulation

Fluidized bed granulation is a process which involves conversion of atomizable liquids such as suspensions and emulsions into free-flowing granular solids by the inclusion of different processes such as wetting, drying, particle size enlargement and homogenization into a single step of processing. (Morl et al., 2004).

The instrument used for Fluidized bed granulation is a fluidized bed agglomerator, the same instrument which is used for agglomeration (SDA). A fluidized bed of powder is formed when warm air is passed through a seed material placed at the bottom of agglomeration chamber. This causes intense movement of the particles inside the agglomerator and hence a fluidized bed is formed. These instruments can be run at top spray or bottom spray mode.

The process involves three stages:

1) Using a stream of air to fluidize the bed.
2) Adding the flavor emulsion in the agglomerator to form flavor encapsulates
3) Passing the air to dry the particles.

Flavoring emulsions are atomized by the nozzle systems integrated in the fluidized bed plant. Passing the hot air through the sprayed emulsion causes evaporation, which produces granulation seeds. These fluidized particles are continuously sprayed with emulsion, which results in formation of liquid and solid bridges between the individual particles and hence agglomerates grow as the spraying is continued. The liquid dries on the seed material and loads it with the active substance. A dust-free, easy flowing agglomerate of excellent solubility is formed. (Babic and Neuhaus-Neotec, 2015; Burger et al., 2006). Residence time of the emulsion in fluidized bed is controllable and hence, the process can be continued until the required particle size is obtained. (Burger et al., 2006)

Burger et al. (2006) have investigated the application of fluidized bed granulation for production of moisture and oxygen stable encapsulates. Encapsulates were comprised of an active compound which was encapsulated in varying amounts of high molecular weight film forming carbohydrates, maltodextrins and sucrose. Aqueous emulsion of the above carrier solids along with the flavor compound was made and sprayed into a fluidized bed agglomerator (instrument similar to one used in research). Fluidized bed granulation can be operated in continuous or in batch-wise processing with several products for many different applications. Fluidized bed granulation was carried out by placement of inert seed material inside the instrument followed by spraying of emulsion of the active compound. Inlet air temperatures in the range of 60 to 100° C were used and resulted in formation of active compound encapsulated in glassy state carbohydrate matrix. As residence time of product inside agglomerator was a parameter that could be controlled and hence emulsion spraying could be continued till the required particle size is obtained. For the research presented in this thesis, a bottom-spray batch process was studied. Although, research suggests that continuous granulation process can be preferred to ensure maximum uniformity of the product. This process can be applied for encapsulation of food flavors, pharmaceutical and surface-active compounds. (Burger et al., 2006).

In their patent, Nagao and Nakamura (2009) discuss a process in which sugars such as maltose and trehalose were mixed with flavor material (about 10:1 ratio) to obtain an “absorbed flavor”. The flavor was passed between compression rollers to obtain plated

matter. This plated material was ground to obtain a granulated flavor. The plated matter could be cooled before grinding. The flavors could be roll molded for application in chewing gums. The flavors obtained using this method offered excellent storage stability and good physical characteristics such as hardness and uniform large particle size. The process offers lower run time when compared to freeze drying and particles with larger size and better solubility than spray drying.

De Ross et al. (2000) described a multi-stage spray drying unit. In the first stage, flavor emulsion was dried and in the next stage; fluidized bed agglomeration took place. Encapsulates that were obtained had a particle size in the range of 100-400 μm. A low moisture content was obtained although flavor retention was lower than single stage dryers. Absolute density was comparable to that of SDA samples and lower than that of FBG samples which were produced as a part of this research.

Chida (2012) discussed a method for preparation of granules by placing flavor inside an agglomerator and then spraying binder liquid until the moisture content reached a critical level under fluidization and then drying the mixture by passing the hot air through the product. The procedure is similar to agglomeration. Results were not discussed in this patent.

Looft et al. (2015) used a granulation process similar to the research presented in this thesis. Tea dust was used as seed material and an emulsion containing water, maltodextrin, gum arabic and tea flavor was injected into the fluid bed and granulation was carried out for 40 mins. Agglomerates with particle size in the range of 500-700 μm were obtained. Microcrystalline cellulose, pea starch and tea leaves can also be used as seed material. Sensory studies were performed to determine the best ingredients for tea agglomeration. Microcrystalline cellulose, cellulose fibers, pea starch and extracted and dried black tea leaves were found to have no effect on flavor profile while sucrose and non-extracted tea leaves were found to have an effect in flavor profile of the product. Overall, the authors found that the process had a lot of flexibility regarding using seed material ingredients and flavor compounds that can be encapsulated.

Schleifenbaum et al. (2016) used a one-step fluidized bed encapsulation procedure like the fluidized bed granulation process used in this research. Emulsions with similar

components (maltodextrin and modified starch as carrier solids + water + flavoring) but with different weight ratios of carrier solids were used to make emulsion of different flavors such as strawberry and chicken. Nitrogen gas at about 140°C was used for fluidization and the granules made by this process can be coated further in same instrument by other flavor emulsions or fats using similar encapsulation conditions. The said encapsulates were found to have applicability and usage in products such as biscuits, ice cream, fruit jellies and tablets. About 25% flavor load was used and it resulted in product with 60 -90% flavor retentions.

Benelli et al. (2014) discussed a fluidized bed encapsulation method for Rosemary extract which was similar to the fluidized bed granulation process used in this research. The encapsulates prepared by fluidized bed encapsulation method were compared with encapsulates prepared by spray drying for parameters such as shelf life, polyphenol retention and bulk density. Cassava flour and sugar pellets were used as seed material. It was observed that fluidized bed granules possessed better flowability and higher retention of polyphenols than spray dried encapsulates. The higher retention was postulated to be due to different drying mechanisms for both processes. In spray drying process, the product dries due to direct contact with hot drying air, which results in higher temperature of droplet. Product residence time is higher in case of granulation and in case of fluidized bed process, it was observed that the coating on core material gets renewed continuously; which might result in lower loss of volatiles. Fluidized bed granules had larger particle size, higher bulk density and better flowability. Spray dried powders were observed to be sticky and had a tendency agglomerate. Use of sugar pellets as a seed material gave particles with smoother surface and spherical shape than cassava flour. Polyphenol retention of up to 100% was obtained using fluidized bed encapsulation.

Chapter II: Objective & Hypotheses

A. Objective

To study how encapsulates obtained from fluidized bed granulation (FBG) compare against encapsulates made using spray drying (SD) and spray drying + agglomeration (SDA)

B. Hypotheses

1) Fluidized bed granulation (FBG) will yield product with longer shelf life (reduced oxidation) as compared to spray drying (SD) and spray drying +agglomeration (SDA).

2) Fluidized bed granulation (FBG), a single step process, will yield product with larger particle size as compared to spray drying and spray drying +agglomeration (SDA).

3) Fluidized bed granulation (FBG) will yield product with higher oil retention as compared to spray drying and spray drying +agglomeration (SDA).

4) Agglomeration will yield product with a higher density than spray dried orange oil.

Chapter III: Materials & Methods

A. Chemicals

ACS grade acetone (Sigma Aldrich Inc., St. Louis, MO, USA) was used for gas chromatographic analysis (sample preparation). 4-heptanone (Sigma Aldrich Inc., St. Louis, MO, USA) was used as the internal standard for all GC analysis. Anhydrous methanol (Avantor, Center Valley, PA, USA), anhydrous sodium sulfate (Fisher Scientific, Fair Lawn, NJ, USA), and the chemicals comprising the pyridine free reagents kit (Photovolt Instruments Inc., Minneapolis, MN, USA) for the Karl Fischer titration system were used for Karl Fischer moisture analysis. Magnesium chloride hexahydrate salt was used to make saturated salt solutions for the adjustment of sample relative humidity (Sigma Aldrich, St. Louis, MO). Single fold peel Orange oil (Firmenich, Princeton, NJ, USA) was used as the encapsulation load material. MaltrinM-150™ and Capsul™, (octenyl succinic anhydride, OSAn, substituted modified starch) (Ingredion, USA) were used as carrier materials.

B. Preparation of Orange Oil Emulsion

The carrier materials (MD-150 and Capsul) were dissolved at ambient temperature in deionized water and mixed using a high shear mixer (Greerco Corp., Hudson, NH, USA) until complete dissolution. This mixture was allowed to stand overnight and immediately before spray drying, orange oil was added to carrier solid + water mixture and was mixed using the same high shear mixer (Greerco Corp., Hudson, NH, USA) for 15-20 mins. The composition of emulsion used was as follows:

Table 1.Components of the orange oil emulsion used for encapsulation.

Components	weight (g)
Capsul™	360
Maltodextrin 150	3240
Water	2945
Orange oil	900 (25% by weight of carrier solids)

High solids content of emulsion was chosen as it has been observed to have a positive effect on flavor retention during spray drying as high solids content requires minimum drying to assist formation of surface film. Formation of drying film results in slowing of volatile diffusion. Slowing of volatile diffusion means less volatile loss during processing, hence more core material/volatile retention. (Anandaraman, Bangs and Reineccius, 1982)

C. Spray Drying

An APV spray dryer was used in this study. About 7445 g of emulsion was sprayed. The initial inlet temperature was set at 200 ℃ and exit temperature was set at 100 ℃. The evaporation capacity of the dryer is approximately 15kg/h under these air temperatures. High speed centrifugal atomization was used.

Dimensions:

Height of the drying chamber –162.6 cm

Diameter of chamber – 124.5 cm

D. Agglomeration

Spray Dried orange oil was agglomerated in 3 different batches with different run times. (20, 30 and 60 mins).

Equipment - Glatt GmbH Systemtechnik D-01277– GPCG 1- Bottom spray Wurster.

Mesh size used for bottom filter – 20μm

Dimensions – 1.21 m (height) x 0.31m(diameter)

Inlet Temperature – 80°C

Exit Temperature range – 47°C to 54°C

Product Temperature range – 44°Cto 50°C

E. Fluidized bed granulation

Orange oil emulsion with 55% solids content was sprayed in the agglomerator described above. About 200 g of Maltodextrin was used as seed material. Four different batches with different run times were carried out – 25 mins,35 mins,40 mins and 45 mins.

F. Storage of encapsulated powders

Encapsulated samples were kept in desiccator for up to four weeks to have all powders be at a fixed water activity (0.33). The desiccators were wrapped with aluminum foil and kept in dark storage chamber for minimizing light exposure and saturated Magnesium chloride hexahydrate solution was used to obtain relative humidity of 33% in the desiccator. Immediately after spray drying, approximately 15 g of each powder was placed in the RH desiccator to begin the storage study and desiccators were kept in an incubator (Memmert HPP 260). Storage temperature was 35°C. The method was adopted from Anker and Reineccius (1988).

G. Particle Size of encapsulated powders

Particle size is one of the parameters to determine the efficiency of the agglomeration and granulation process. Higher increments in particle size of the spray dried product means the agglomeration process is effective. As larger particle size also denotes better flowability, dispersibility and better handling properties. (Buffo et al., 2001).

To determine the average particle size of the spray dried powders, a Horiba LA-960 Laser Particle Size Analyzer (HORIBA Scientific, Edison, NJ, USA) was used. In this instrument, particle size is determined by measuring the diffraction pattern formed by passing laser light through powder. The diffraction pattern is then treated by light scattering theory to calculate the particle size distribution which would have given that diffraction pattern (Dodds,2013).

Approximately 0.5 g of each powder was added to the instrument. Instrument settings that were used were as follows - (Air pressure: 0.15 mPa, Feeder: 100% (Automatic),

Refractive Index: 1.67). **Particle size was analyzed in duplicates.** Mean Particle size (volume mean diameter of powder particles) of the powders were recorded.

H. Moisture Content by the Karl Fischer Method

Water activity influences the physical properties, microbiological viability and growth, sensory properties, stability and phase behavior of foods. Moisture content is defined as ratio of mass of water in a sample to mass of solids in the same sample, generally expressed in a percent value. Although all samples were equilibrated to the same a_w (0.33), it was of interest to measure water content of the samples.

The moisture content of water activity adjusted samples was determined by the Karl Fischer method using a Metrohm KF 756 Aquatest CMA instrument (Photovolt Instruments Inc., Minneapolis, MN, USA). Approximately 0.20-0.25 g of sample was weighed into 20-mL headspace vials. Approximately 20 g of anhydrous methanol was weighed into the vial that contained the encapsulated material and the vial was capped and allowed to equilibrate at overnight using a shaker. Approximately 1 mL of sample mixture was injected into the Aquatest CMA instrument and the Aquatest output reading was used to calculate the moisture content on a dry basis according to the manual instructions. Methanol blanks were also prepared by weighing 20 g of methanol and were measured to correct for residual moisture in the solvent. All samples were analyzed in **duplicates**, including blanks.

I. Particle Density by Nitrogen Pycnometry

Density is defined as the ratio of mass of a material and its volume. Density is reported in g/cc or kg/m3 units. Particle density is defined by ratio of the mass of powdered particles and volume occupied by said particles. Particle density of powders was determined with a multi-pycnometer (QuantaChrome Corp., Boynton Beach, FL). This instrument is specifically designed to measure the volume of powders. Archimedes principle of fluid displacement is employed to determine the volume of the sample. Non-reactive gases such as Nitrogen and Helium are used as the fluid material and

Helium can enter the smallest pore present in the sample due to smaller atomic size than other gases. (Buffo et al., 2001).

The pycnometer was calibrated according to the manual instructions. Large sample cylinder(cell) was used for determination of density. To determine the specific volume, approximately 15 to 20 g of encapsulated material was weighed into the sample cell of a Quantachrome Multipycnometer (Quantachrome Corporation, Boynton Beach, FL, USA). Density analyses were performed **in duplicates.** True powder volume is calculated by following equation

Vp =Vc -Vr {(P1/P2)-1}

Here, Vp – Powder Volume Vc – Large Sample Cell Volume Vr – Large Reference Volume.

Density is Calculated by following Formula- Density = Mass of product inside sample cell/ Vp.

J. Clevenger Distillation – Total Oil Content

Flavor retention can be determined using Clevenger distillation. Clevenger distillation gives us total oil content, i.e. a measure of flavor retention during processing. It is a traditional method which can be used to determine the retention of water- insoluble materials such as essential oils. Peak areas obtained from a gas chromatograph can also be used to calculate the limonene oxide content of the sample. (Anandaraman and Reineccius,1986).

Encapsulates (20 g) were dissolved in 200 mL of distilled water in a 500-mL round-bottom flask. Then, 0.1 mL of vegetable oil (Market pantry 100% Canola oil) was added to the flask as an antifoam and the Clevenger apparatus was connected to the flask with a water-cooled condenser on top.

The distillation was carried out for 3 hrs. Water and oil layers were allowed to separate in the collection arm and then the volume of distilled oil was read directly from the collection arm. The volume of oil was converted to mass by multiplying by the density of the oil (0.85 g/mL) as determined gravimetrically at 25^{o}C.

The volatile oil retention (overall aroma retention) during drying was calculated as follows:

Volatile oil retention % = [(Measured oil content)/ (theoretical oil content)] X 100%

Powder needed for each test = 20 g X 2 (duplicates) = 40 g.

Flavor retention calculations were also performed using peak areas obtained from GC chromatographs for week 0 (pre oxidation storage) samples. Method for sample preparation and method operation is described in section K: Limonene Oxidation by Gas Chromatography (GC) of this thesis. Value for grams of limonene per gram of sample was calculated using standard equation constructed for limonene. The said value was compared with the theoretical oil content value and yield was calculated as follows:

Volatile oil retention % = [(Oil content obtained using GC peak area)/ (theoretical oil content)] X 100%

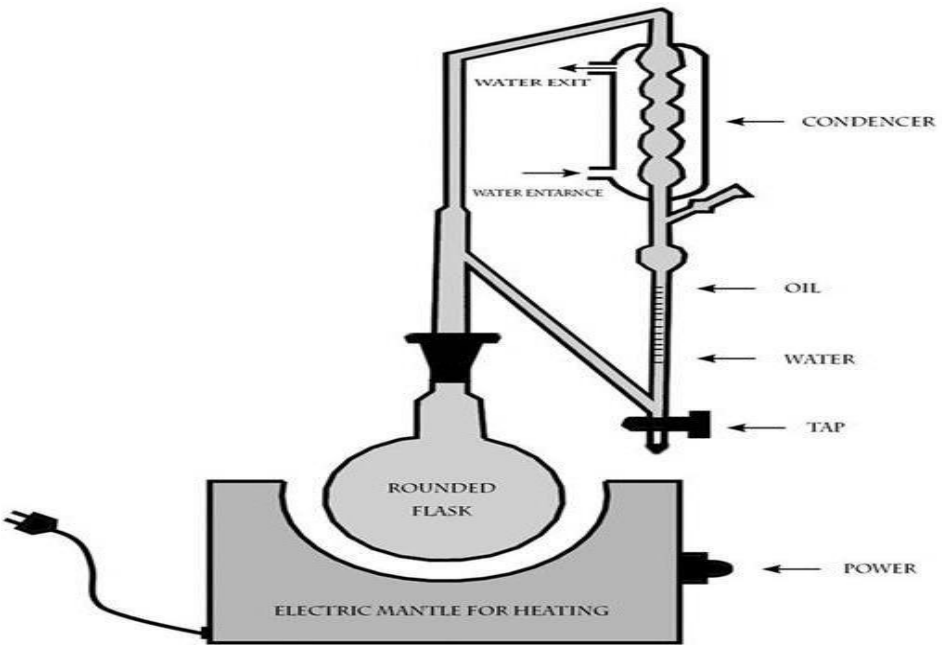

Figure 4. A simple diagram demonstrating a Clevenger distillation apparatus. From Ismaiel et al. (2016)

K. Limonene Oxidation by Gas Chromatography (GC)

Gas chromatography is a type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. GC is widely used in flavor research as it is having excellent separation powers and high sensitivity. (Reineccius, 2004).

Flavor oxidation was analyzed using Gas Chromatograph using a flame ionization detector. Flame ionization detector is used to due to its low detection limits and ability to analyze organic compounds and hydrocarbons. (Harris, 1999). Limonene is important chemical component of orange oil (about 95% limonene). Limonene oxide and carvone are the first compounds that are formed during limonene oxidation. (Anandaraman and Reineccius, 1986). As carvone has lower rate of formation than limonene oxide (Anandaraman and Reineccius, 1986), limonene oxide concentration was chosen as the measurement parameter for this Oxidation study.

Other methods can also be used to study flavor oxidation such as peroxide value determination. Two methods can be used to determine peroxide value – iodometric method and colorimetric method (using Titanium dioxide). (Ananadaraman and Reineccius, 1987). The iodometric method is less suitable for analysis of orange oil encapsulates as it gives lower peroxide values as Iodine which is used for the analysis can be occluded by starch molecules and hence less Iodine is available for reaction with peroxides and hydroperoxides, which results in incorrect and lower determination. (Anandaraman and Reineccius,1987). A colorimetric method using Titanium dioxide is more accurate for analysis of orange oil encapsulates, although it can only detect hydroperoxides which are formed during early stages of oxidation and hence, is a method more suitable for determination of early stage oxidation in samples.

Limonene oxide was calculated as the sum of the peak areas of its two major isomers and the oxidative stability of limonene was expressed as the ratio of peak areas of the sum of limonene oxide isomers to limonene (defined as mg LO/L ratio in this research), thus the information could be interpreted as mg of limonene oxide formed per g of

limonene. Flavor oxidation can be triggered by a variety of factors such as water activity, atmospheric contact and oxygen diffusion. (Reineccius and Yan, 2016).

Structures of limonene and limonene oxide:

A – limonene B – cis limonene Oxide C – trans limonene oxide

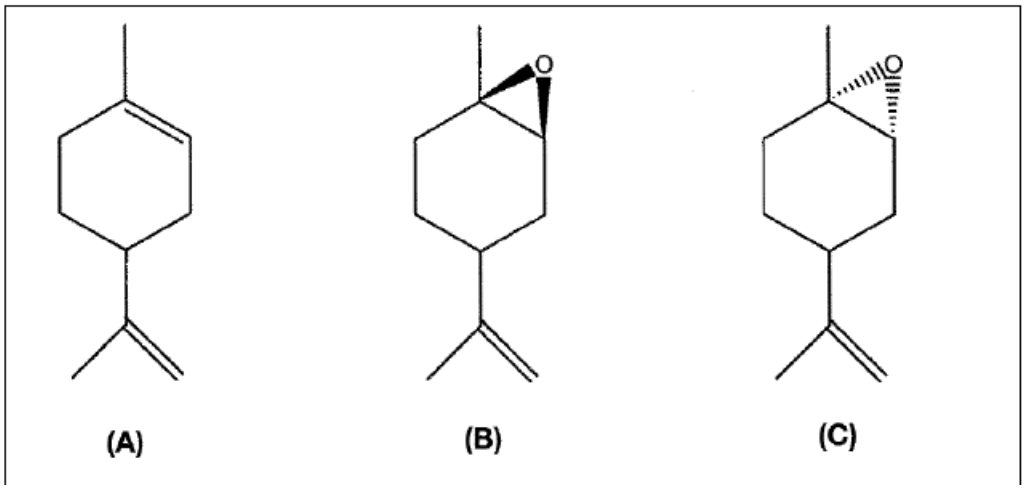

Figure 5. Chemical structures of limonene and major limonene oxide isomers. Adopted from Determination of Limonene Oxidation Products using SPME and GC–MS by Sonchik-Marine and Clemons (2003).

To determine the stability of limonene, the encapsulates are analyzed using an acetone "extraction" method.

The steps are as follows:

1. Encapsulates (1.5 g) were dissolved in 8.5 mL of water and mixed using a vortex mixer.

2. The mixture (1 mL) was transferred to another vial, with 4 mL of acetone solution added with constant stirring.

3. After mixing, the mixture was allowed to settle for one hour and a portion of the supernatant was transferred to 2 mL autosampler vials and loaded into a HP7673A automatic sampler (Hewlett-Packard, Wilmington, DE).

4. About 2 μL of each extract was automatically injected in split mode into a HP 5890 series II GC equipped with a HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm) (J&W Scientific, Folsom, CA) and a flame ionization detector (FID).

All the samples were analyzed in duplicates.

The operating conditions were as follows:

head pressure - 12 psi

initial oven temperature 50°C, increased at 10°C/min to 140°C with 5 min hold, and then at 30°C/min to 280°C with 5 min hold

Injection port 220°C

Data collection and peak area integrations were performed using the Chemstation software.

Limonene oxide is reported as the sum of its two major isomers. The oxidative stability of limonene is expressed as the ratio of peak areas of the sum of limonene oxide isomers to limonene, thus the information could be interpreted as mg of limonene oxide formed per g of limonene.

For each sample, 5 GC runs were performed. (1st one after water activity equilibration, one analysis each week for 4 weeks in storage.) Each sample was analyzed in **duplicate**. Hence, 10 replicates were obtained for each sample. For each sample, one data point was subtracted from its duplicate. All duplicate differences were added, and average was taken. The average can be expressed as the average deviation. An error bar equal to average deviation was added on Limonene oxide plots (figure 10 and figure 11).

Chapter IV: Results and Discussion

A. Particle property analysis

1. Particle Size Comparison

Under the operating conditions that were utilized in this research, granulated samples had the largest particle size values amongst the prepared encapsulates. The larger particle size of the FBG samples can be explained by the continuous spraying of emulsion while processing, which results in continuous drying and film formation on granules (particle growth), as compared to wetting and drying cycles for SDA samples and single time drying operation in case of spray drying. Spray dried samples offered very low mean particle size values (about 60.7 micrometers). Agglomeration of the spray dried orange oil did increase the particle size as expected by Buffo et al. (2001), Reineccius (2004). Particle size data obtained was in the range of 0.1 to 3.0 mm (Burger et al., 2006). Similar data to this research was obtained although emulsion constitution was different and higher inlet air, exhaust air and product temperatures were used in study done by Burger. (1:1 ratio of Maltodextrin: Capsul^{TM} by weight and sucrose was also added in case of some batches.). Looft et al. (2015) also obtained particle sizes in the range of 500-700 μm which is similar to the range obtained for FBG samples (449 – 628 μm.). Benelli et al. (2014) also obtained rosemary extract encapsulates with larger particle size than spray drying products by using fluidized bed granulation. Mean particle sizes obtained in their results were similar to particle sizes obtained by FBG in this research (611 to 645 Mm).

Following abbreviations are used for graphs:

Spray Dried – Spray drying

Agg run 30 mins - Agglomeration (SDA) run 30 minutes.

Agg run 60 mins - Agglomeration (SDA) run 60 minutes.

Agg run 90 mins - Agglomeration (SDA) run 90 minutes.

Gran 25 mins - FBG run 25 minutes.

Gran 35 mins - FBG run 35 minutes.

Gran 40 mins - FBG run 40 minutes.

Gran 45 mins – FBG run 45 minutes.

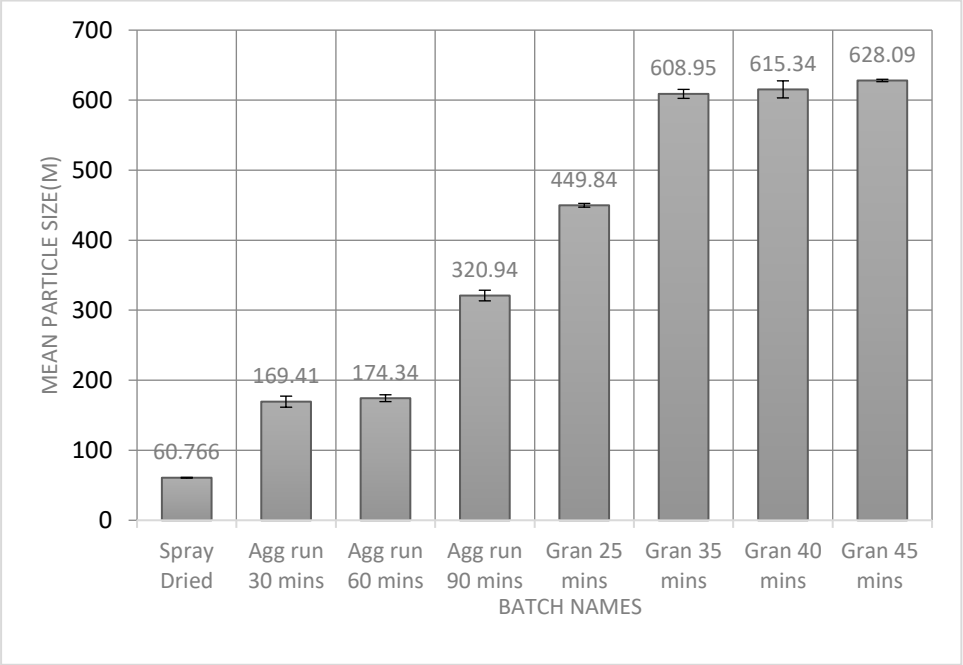

Figure 6. Influence of process and process times on Mean Particle size (mm) of different samples (values denote an average value of the mean particle sizes obtained for the duplicates).

The highest mean and median particle size values obtained by agglomeration are still less than 50% than that of granulation samples. Hence, it was observed that granulation can produce a larger particle size than spray drying (SD) and agglomeration (SDA) samples in equivalent (or shorter) processing times. Particle size distribution data for 3 batches (spray drying, SDA 90 mins run and FBG 45 mins run) is shown on page 23. It can be observed from the graphs that FBG sample had more uniform particle size distribution when compared to SDA and spray drying samples as the distribution graph has a single, clearly defined peak in contrast to less finely defined peak of spray drying sample and two visible peaks obtained for SDA sample.

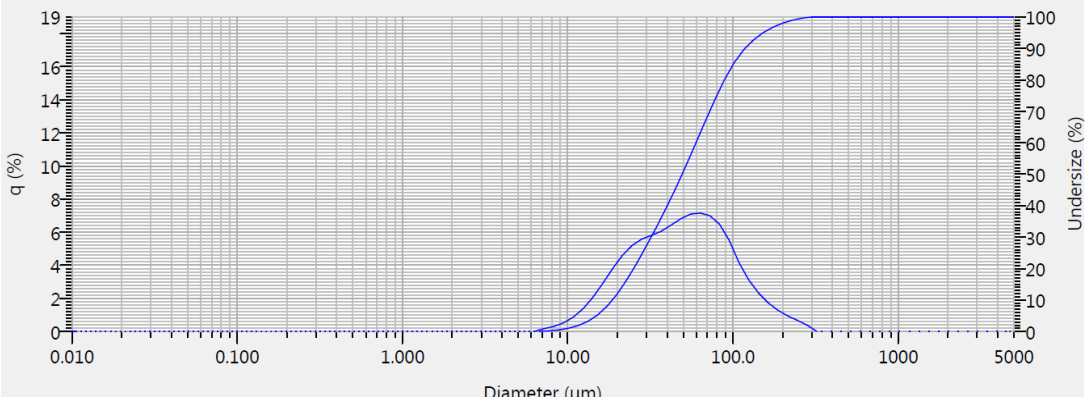

Figure 7. Particle size distribution for spray dried sample.

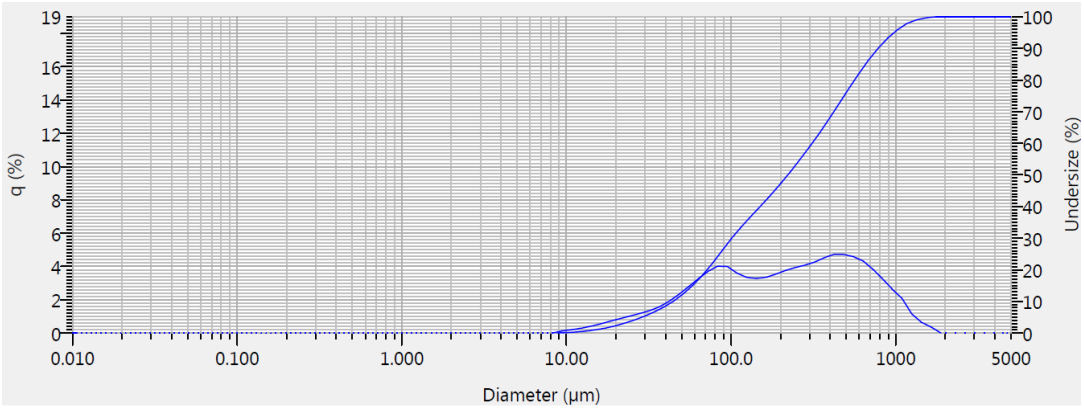

Figure 8. Particle size distribution for agglomeration 90 mins run.

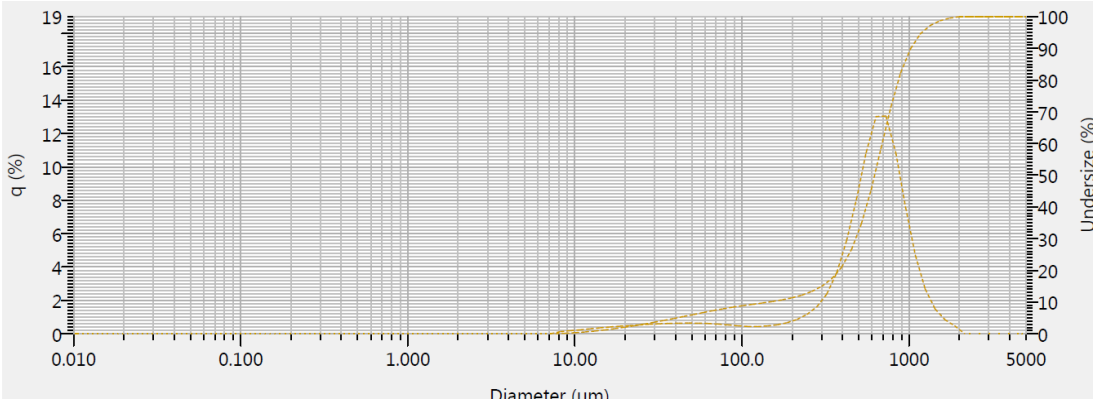

Figure 9. Particle size distribution for granulation 45 minutes run.

2. Moisture Content

Under the operating conditions used in this research, comparison of % moisture content between different samples shows that granulated samples have least moisture content while SDA samples have highest moisture content of all samples prepared. Since, final

moisture content of the product is a function of equipment operation and not dependent on process, it is not an accurate estimate of process. Similar final moisture content can be obtained by controlling wetting and drying cycle period of the agglomeration process. As it is difficult to get an accurate and quick estimate of moisture using a standardized method while process is being run, no comparison can be made about efficiency of the process. Data is included in appendix -table 20.

3.Particle density comparison

It can be observed from Figure 7 below that fluidized bed granulation produced samples with highest particle density followed by fluidized bed agglomeration. Spray dried Orange oil emulsion had the lowest particle density compared to other samples. In the case of agglomeration and granulation, density values were observed to increase with increased run time of the process. A difference was observed between 35 mins run time granulation (1.26 g/cc) and 40 mins run time granulation (1.4 g/cc) and, between 30 min agglomeration run (1.07 g/cc) and 60 mins agglomeration run (1.16 g/cc).

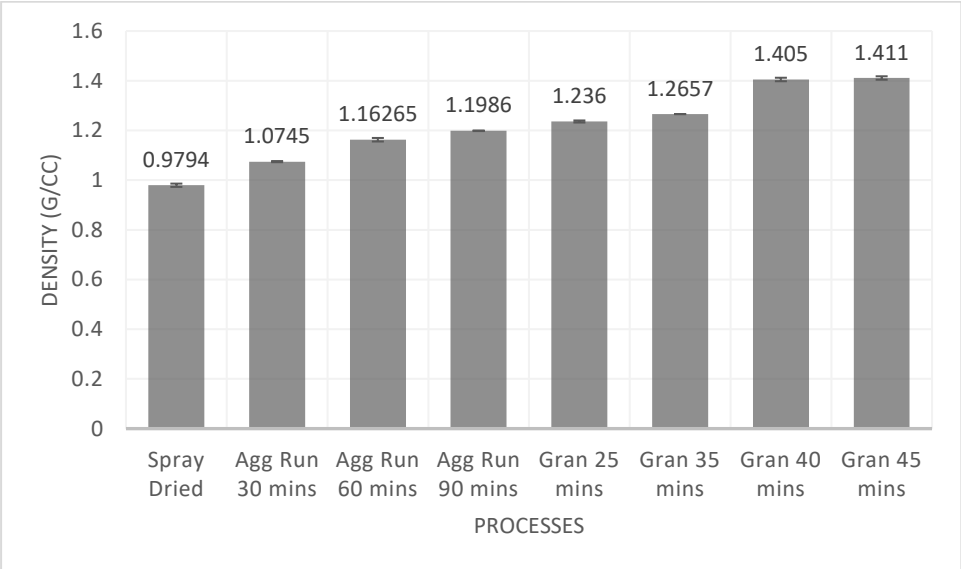

Figure 10. Effect of processes and process times on particle density of spray dried (SD), agglomeration (SDA) and fluidized bed granulation (FBG) encapsulates.

It was also found that agglomeration increased the density of the spray dried orange oil up to 22%. Similar density values in the range of 0.98 to 1.21 g/cc were reported by Buffo, although his carrier solid compositions were different. (Buffo et al., 2001).

B. Shelf Life Study

1. Effect of processing on oxidation of limonene

Immediately after processing, it was observed that the granulation batch (40 mins run time) which was ran at a higher operating temperature than that of other 3 granulation batches; had the highest limonene oxide content at week zero, followed by spray dried and SDA samples. These values indicate the damage that is done during the processing step. Anantharamakrishnan and Reineccius (2017) also reported higher limonene oxide formation due to exposure to heat during processing in their research.

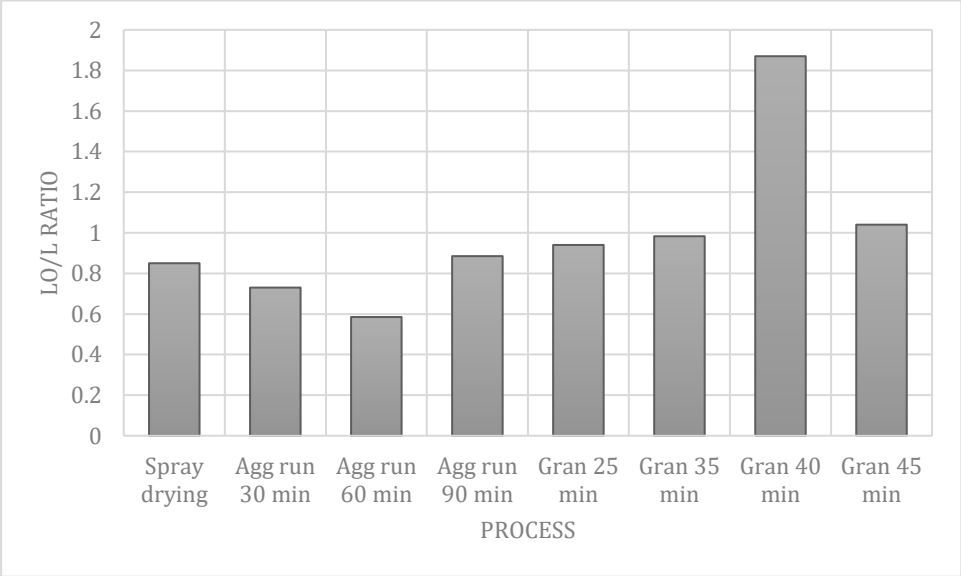

Figure 11. Initial (After production, before water activity adjustment) LO/L (After processing and before storage) for all samples.

2. Effect of storage on the oxidation of limonene

It can be observed from the Figures 8(all samples),9(SD and SDA) and 10(FBG) below and table that the spray drying samples had the highest amount of limonene oxide formed during the storage. It was closely followed by Agglomerated samples. The least

formation of limonene oxide and hence least extent of oxidation was observed in the granulation samples. For FBG batches, granulation batch with 40 mins run time had highest amount of oxidation observed as the batch was run at higher operating temperatures than other batches of FBG. Anantharamakrishnan and Reineccius (2017) confirmed in their research that products exposed to higher heat have higher extent of oxidation. This demonstrates that the temperature used in granulation will influence stability to oxidation.

After 4 weeks of storage, the largest quantity of limonene oxide content was observed to be in spray dried orange oil, followed by agglomerated orange oil encapsulates. Granulated samples had lowest net limonene oxide content and LO/L ratio after 4 weeks of storage, with again product from batch ran at higher temperatures, had more limonene oxide formation. Granulation batch which was ran for 25 mins had the least limonene oxide content after completion of the study, while batch with run time of 35 and 45 mins had no significant difference between final limonene oxide content.

As spray dried orange oil encapsulates have lower density and due to lower density more particles are present in a given unit volume as compared to agglomerated and granulated products. Hence, more surface area is available for oxidation per unit mass/volume. This results in higher amount of exposure towards air and other agent which may cause oxidation of orange oil such as moisture and light. Hence, more oxidation can take place inside the spray dried sample resulting in higher limonene oxide content during the end of shelf life study. Also, granulated products had larger particle size as compared to agglomerated products; hence it can be seen that extent of oxidation is less due to lower surface area per unit mass in granulated products than in agglomerated products. Also, granulation process was carried out at lower temperatures as compared to spray drying, that may also contribute towards lower oxidation during storage time. Soottitantawat et al. (2004) reported that an increase in particle size decreased the flavor oxidation in spray dried samples.

It can also be observed that in the case of granulation samples, longer run time may contribute towards higher oxidation as product is exposed to heat for a longer time. From Figure 10, it can be observed that batch with 25 mins run time has least limonene oxide formation after 4 weeks of storage as compared to other granulation batches. In

case of agglomerated samples, similar trend is observed for 30 mins and 60 mins run time batches although the larger particle size in 3rd batch (90 mins run time) seems to have contributed towards lower limonene oxide content than compared to other two agglomeration runs (refer figure 13).

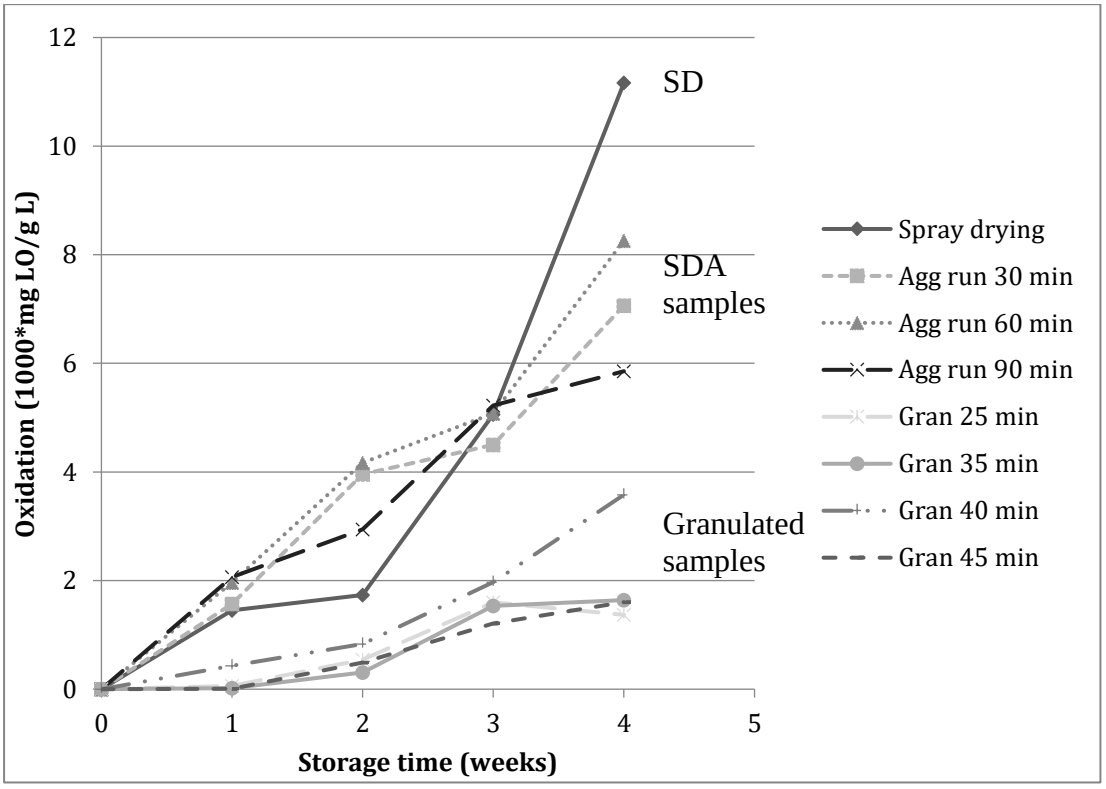

Figure 12. Effect of the storage time on oxidation (LO/L ratio) for all samples.

The ratios of sum of limonene oxide isomer peak areas (LO) and limonene peak areas (L) were calculated for all samples over 4 weeks of storage. This value is defined as oxidative stability of the sample. Granulated samples showed smaller ratios and spray dried sample showed highest ratios; with agglomeration samples nestling between the two.

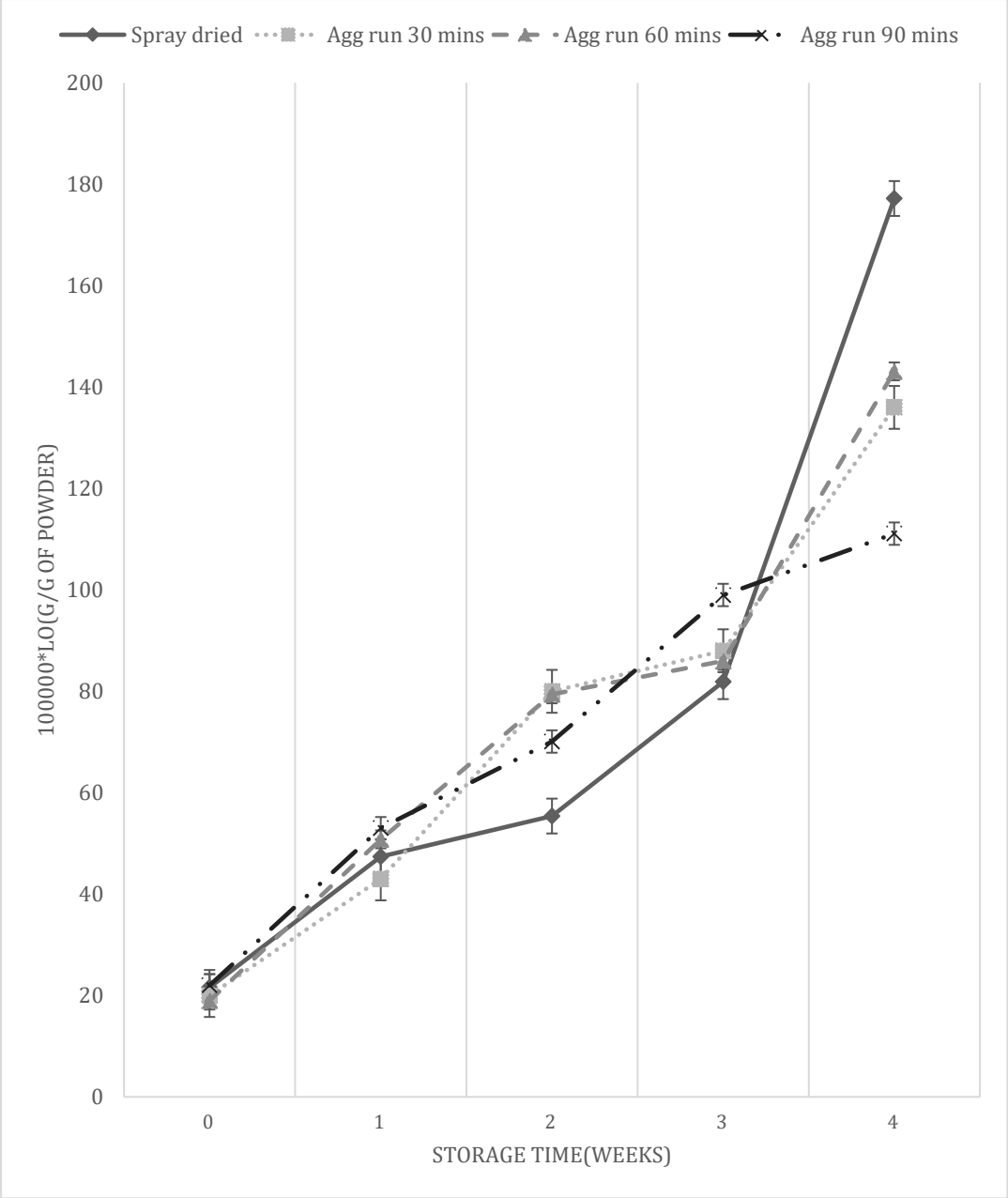

Figure 13. Effect of storage time on limonene oxide content of the SD and SDA samples.

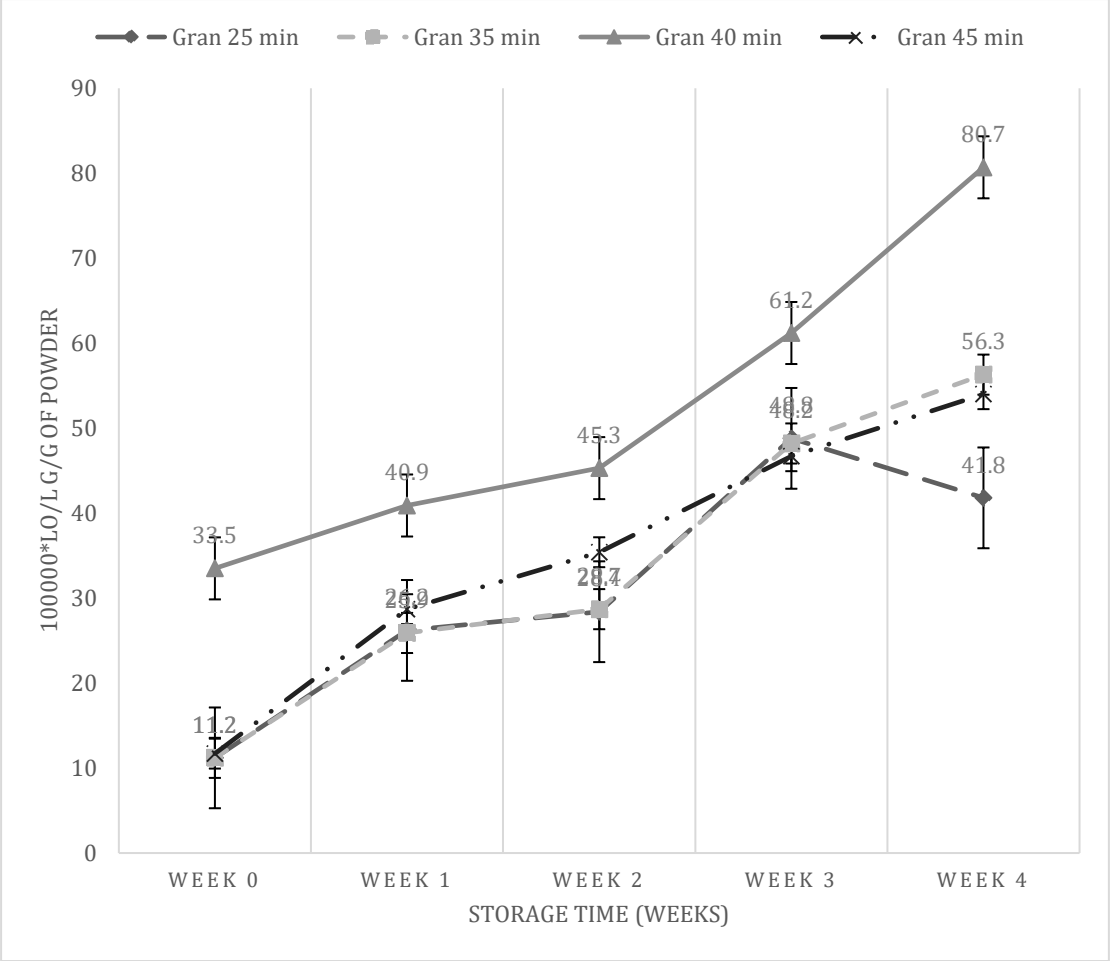

Figure 14. Effect of storage time on limonene oxide content of the granulation samples.

It can be observed from the figure 8 that the spray dried sample (SD) had the highest increase in the amount of limonene oxide formed during the storage. It was closely followed by Agglomerated samples. Least formation of limonene oxide and hence least extent of oxidation was observed in granulation samples, except 40 mins run time batch; which was ran at a higher temperature than other batches. Highest amount of initial limonene oxide formation (in 1st week) took place in 40 mins granulation batch, followed by spray drying and agglomerated batches. Less initial limonene oxide formation was observed in other 3 granulation batches which were ran at lower temperatures.

It can also be observed that higher run time may contribute towards higher oxidation as product is exposed to heat for a longer time. Anantharamakrishnan and Reineccius (2017) found out in case of spray dried powders that increased exposure to heat and exposure of samples to higher temperatures increases the extent of oxidation. Although in case of agglomerated samples, **higher particle size** in 3rd batch seems to have contributed towards lower limonene oxide content than compared to other two agglomeration runs.

As the storage studies were conducted, it was observed that the rate of limonene oxide production was highest in spray dried sample and lowest in all granulated samples, resulting in highest final limonene oxide content after 4 weeks for the spray dried (SD) sample.

C. Effect of processing on the retention of orange oil.

Table 2. % theoretical flavor weight in powder for all samples.

Process	% theoretical flavor weight
Spray drying	25.00
Agg run 30 mins	25.00
Agg run 60 mins	25.00
Agg run 90 mins	25.00
Gran 25 mins	12.18
Gran 35 mins	15.03
Gran 40 mins	15.03
Gran 45 mins	16.30

From the figure 11 above, it can be observed that flavor content in final product is different for different processes. Difference in % theoretical flavor weight between FBG and SD + SDA samples is due to additional seed material used in the FBG, which increases the carrier solid content in the final product and hence, decreases % flavor load.

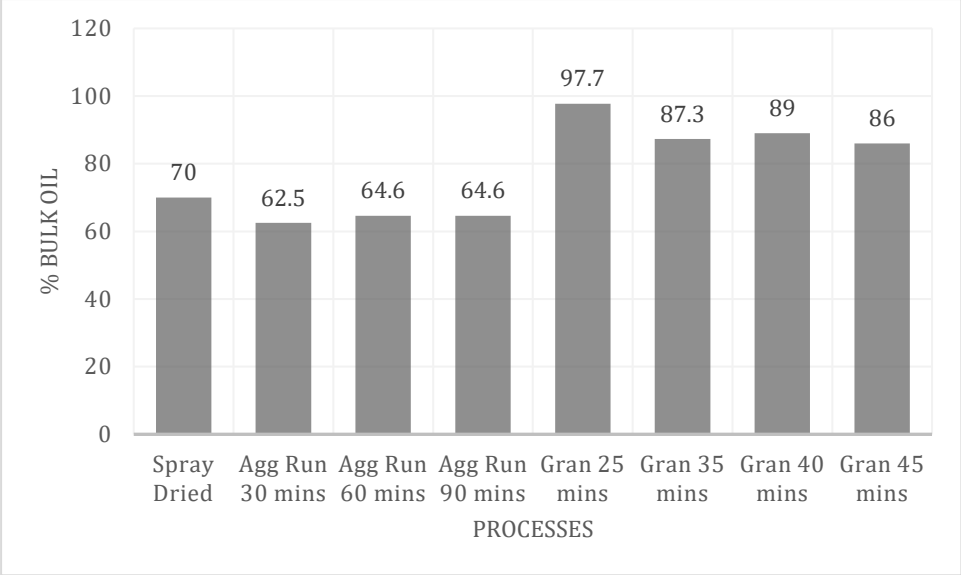

Figure 15. Effect of processes and process times on % oil retention calculated using Clevenger distillation.

It can be seen from figure 15 that granulation samples had the highest oil retention. Agglomeration samples were observed to have lowest retention followed by spray drying. Higher oil retention compared to spray drying and SDA can be explained by lower operating temperatures of the granulation process as lower operating temperature corresponds to lower loss of volatile matter. Higher retention in spray dried samples as compared to SDA samples has also been reported by Buffo et al. (2001). Also, usage of Maltodextrin as seed material increases the effective solids content of the final encapsulates, which contributes towards limiting convection currents within drying droplets and slows volatile diffusion towards particle surface; (Anandaraman, Bangs and Reineccius, 1982) which might explain higher flavor retention. Benelli et al. (2014) explained the lower flavor retention due to product drying due to direct contact with hot air, which results in higher temperature of core droplet and hence some loss of volatiles is possible.

Table 3. Effect of processes and process times on % oil retention calculated using GC data.

Process	% oil by Clevenger (Dry basis calculation)	%oil by GC analysis
Spray dried	70	60
Agg Run 30 mins	62.5	53
Agg Run 60 mins	64.6	54
Agg Run 90 mins	64.6	53.5
Gran 25 mins	97.7	53
Gran 35 mins	87.3	55
Gran 40 mins	89	57
Gran 45 mins	86	59

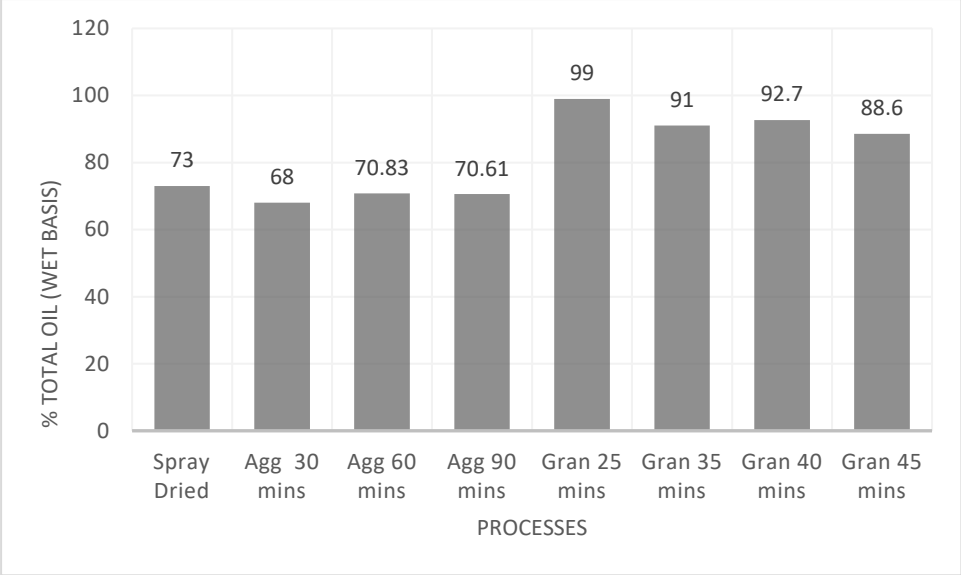

Figure 16. Effect of processes and process times on % oil retention calculated using Clevenger distillation. (calculated on wet basis).

Figure 16 shows the % oil retention calculated using wet basis (subtracting moisture content from powder weight used for analysis). Higher values were obtained using wet basis calculation. Similar trend of the flavor retention values was observed to be followed with FBG samples which show highest retention values and SDA samples showing the lowest retention values.

Calculation of % retention by GC data

Comparison of Retention values by GC Data and by Clevenger Distillation data (refer table 3) showed that values obtained by Clevenger distillation were higher than the values calculated using peak areas obtained from chromatographs. About 9-10% difference was observed. The trend that was observed was different when compared to Clevenger distillation data; spray dried (SD) sample giving highest retention values and SDA samples offering lowest retention values. Agglomeration run 1 had lowest retention value by both methods. This may be due to higher processing temperatures and hence more loss of flavors.

Chapter V: Conclusions

As proposed in hypothesis 1, it was observed that granulated products were observed to have better shelf life as compared to SD and SDA samples. Hence, hypothesis 1 was concluded to be true for the operating conditions utilized in the research. SDA samples were observed to have better shelf life when compared to SD samples.

As proposed in hypothesis 2, it was observed that large particles can be obtained via FBG than those were produced by either SD or SDA. Hence, it was concluded that hypothesis 2 is true for the processes under the operating conditions utilized in this research.

As proposed in hypothesis 3, it was observed that, under the operating conditions utilized in this research, FBG had better oil retention results than SDA and spray drying using Clevenger distillation method. Similar result trends were obtained using wet and dry basis calculations for the Clevenger distillation. However, for the flavor retention calculation using GC peak areas, it was observed that SD had the highest flavor retention followed by FBG and SDA encapsulates having lowest retention values. Hence, hypothesis 3 was observed to be true only for Clevenger distillation method.

FBG encapsulates had highest density values followed by SDA. Spray dried encapsulates had the lowest density values. As proposed in hypothesis 4, fluidized bed agglomeration did increase the density of the spray dried sample. Density was observed to be directly proportional to the particle size.

It can be said that fluidized bed granulation is an alternative to more conventional, widespread spray drying+ agglomeration process as it carries out the same work in one step and it produces encapsulates with overall better functional properties than conventional processes.

Chapter VI: Future Scope

Similar research can be replicated using gum acacia + maltodextrin 150 carrier matrix as gum acacia has also proven to be a capable carrier material for spray drying in many previous published research articles as fairly similar results can be replicated.

Also, similar research can be done by using oils such as lemon oil, clove oil and other essential oils. It will be very interesting to see the viability and applicability of this process to a wide variety of core materials. It can be suggested to use for the essential oils as all of them have similar chemical constitution (mostly composed of aromatic hydrocarbons).

One limitation of this research was the only available instrument for granulation was bottom spray agglomerator present in the pilot plant of this department. Babic (2015) has replicated similar research using continuous top spray granulator instrument and product with even better functional properties was obtained. Hence, the above-mentioned research can be replicated using a continuous top spray granulator.

Overall, this process has a lot of potential for growth in food industry.

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Chapter VIII: Appendices

Table 4a. Raw data for oil retention by Clevenger distillation: SD and SDA-30 mins samples.

	Spray Drying Batch.		Agglomeration 30 min run	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Volume of oil in Clevenger's	4.1 ml	4.1 ml	3.1 ml	3.1 ml
Density of sample	0.85 g/ml	0.85 g/ml	0.85 g/ml	0.85 g/ml
Weight of Oil	3.5 g	3.5 g	2.975 g	2.975 g

Table 4b. Raw data for oil retention by Clevenger distillation: SDA-60 mins and SDA-90 mins samples.

	Agglomeration 60 min run		Agglomeration 90 min run	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Volume of oil	3.4 ml	3.4ml	3.4 ml	3.4 ml
Density of sample	0.85 g/ml	0.85 g/ml	0.85 g/ml	0.85 g/ml
Weight of Oil	3.23 g	3.23 g	3.23 g	3.23 g

Table 5a. Raw data for oil retention by Clevenger distillation: FBG 25 mins an FBG 40 mins samples.

	Granulation 25 mins batch		Granulation 40 mins run Batch	
Volume of oil	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	3.3 ml	3.3 ml	3.5 ml	3.5 ml
Density of sample	0.85 g/ml	0.85 g/ml	0.85 g/ml	0.85 g/ml
Weight of Oil	2.8 g	2.8 g	2.975 g	2.975 g

Table 5b. Raw data for oil retention by Clevenger distillation: FBG 45 mins an FBG 30 mins samples.

	Granulation 45 mins run time batch		Granulation 30 mins run Batch	
Volume of oil	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	3.6 ml	3.6 ml	3.4 ml	3.4 ml
Density of sample	0.85 g/ml	0.85 g/ml	0.85 g/ml	0.85 g/ml
Weight of Oil	3.06 g	3.06 g	2.89 g	2.89 g

Table 6. Density measurement by Pycnometer raw data: SD and SDA samples

	Spray Drying Batch.		Agglomeration 30 mins batch		Agglomeration 60 mins batch		Agglomeration 90 mins batch	
	Replicate		Replicate		Replicate		Replicate	
	1	2	1	2	1	2	1	2
P1	17.004	17.001	17.037	16.907	17.006	17.036	17.005	17.005
P2	7.644	7.653	7.484	7.429	7.378	7.401	7.42	7.422
Vp	41.042	40.639	35.525	35.578	33.231	33.511	34.38	34.46
Density (g/cc)	0.9746	0.9842	1.0753	1.0736	1.2037	1.1936	1.1643	1.161
Sample wt	40 g	40g	38.2 g	38.2 g	40 g	40 g	40 g	40 g

Table 7. Density measurement by Pycnometer raw data: FBG samples

	FBG 25 mins batch		FBG 40 mins batch		FBG 45 mins batch		FBG 30 mins batch	
	Replicate		Replicate		Replicate		Replicate	
	1	2	1	2	1	2	1	2
P1	17.674	16.935	17.005	17.005	17.005	17.012	17.192	17.04
P2	7.21	6.913	6.78	6.82	6.78	6.82	7.001	6.959
Vp	20.21	20.3	34.38	34.46	17.8	17.914	19.76	19.79
Density (g/cm3)	1.239	1.233	1.41	1.4	1.41	1.4	1.2656	1.2659
Sample wt (g)	25.04	25.03	40	40	25.1	25.08	25.01	25.05

Table 8. Particle Size Measurement - Spray drying.

Replicate 1	02-07-2017
Median size	49.22707 (µm)
Mean size	61.23553 (µm)
Variance	2058.0 (µm2)
St. Dev.	45.3651 (µm)
Mode size	63.0376 (µm)
Span	Off
Geo. mean size	47.9518 (µm)
Geo. variance	1.2438 (µm2)
Diameter on cumulative % (2)10.00 (%)- 18.5897 (µm) (9)90.00 (%)- 118.2919 (µm)	
D10	18.58966 (µm)
D90	118.29193 (µm)
D (v0.1)	18.58966 (µm)
D (v0.5)	49.22707 (µm)
D (v0.9)	118.2919 (µm)
Replicate 2	02-07-2017
Median size	48.52243 (µm)
Mean size	60.17778 (µm)
Variance	1945.9 (µm2)
St. Dev.	44.1120 (µm)
Mode size	63.0296 (µm)
Span	Off
Geo. mean size	47.2670 (µm)
Geo. variance	1.2410 (µm2)
Diameter on cumulative % (2)10.00 (%)- 18.4101 (µm) (9)90.00 (%)-115.9335(µm)	
D10	18.41008(µm)
D90	115.93351(µm)
D (v0.1)	18.41008(µm)
D (v0.5)	48.52243(µm)
D (v0.9)	115.93351(µm)

Table 9. Particle Size Measurement – FBG 25 mins

Replicate 1	**02-07-2017**
Median size	221.01469 (µm)
Mean size	328.57742 (µm)
Variance	97121 (µm2)
St. Dev.	311.6422 (µm)
Mode size	423.1024 (µm)
Span	Off
Geo. mean size	197.5260 (µm)
Geo. variance	1.6915 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 44.5400 (µm) (9)90.00 (%)- 779.4637 (µm)
D10	44.53996 (µm)
D90	779.46368 (µm)
D (v0.1)	44.53996 (µm)
D (v0.5)	221.01469 (µm)
Replicate 2	**02-07-2017**
Median size	232.21628 (µm)
Mean size	313.30771 (µm)
Variance	75241 (µm2)
St. Dev.	274.3015 (µm)
Mode size	481.9026 (µm)
Span	Off
Geo. mean size	198.3696 (µm)
Geo. variance	1.6226 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 46.2697 (µm) (9)90.00 (%)- 701.6332 (µm)
D10	46.26966 (µm)
D90	701.63318 (µm)

D (v0.1)	46.26966 (µm)
D (v0.5)	232.21628 (µm)
D (v0.9)	701.63318 (µm)
D (v0.9)	779.46368 (µm)

Table 10. Particle Size Measurement - Agglomeration 30 mins

Replicate 1	**02-07-2017**
Median size	162.52747 (µm)
Mean size	177.79512 (µm)
Variance	10424 (µm2)
St. Dev.	102.0988 (µm)
Mode size	186.3953 (µm)
Span	Off
Geo. mean size	147.0203 (µm)
Geo. variance	1.2244 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 63.5356 (µm) (9)90.00 (%)- 308.9195 (µm)
D10	63.53558 (µm)
D90	308.91946 (µm)
D (v0.1)	63.53558 (µm)
D (v0.5)	162.52747 (µm)
D (v0.9)	308.91946 (µm)
Replicate 2	**02-07-2017**
Median size	157.58868 (µm)
Mean size	170.90021 (µm)
Variance	9276.6 (µm2)
St. Dev.	96.3152 (µm)
Mode size	185.9334 (µm)
Span	Off
Geo. mean size	141.7705 (µm)

Geo. variance	1.2205 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 60.5115 (µm) (9)90.00 (%)- 295.8621 (µm)
D10	60.51152 (µm)
D90	295.86212 (µm)
D (v0.1)	60.51152 (µm)
D (v0.5)	157.58868 (µm)
D (v0.9)	295.86227 (µm)

Table 11. Particle Size Measurement - Agglomeration 90 mins

Replicate 1	02-07-2017
Median size	158.41113 (µm)
Mean size	175.00046 (µm)
Variance	11216 (µm2)
St. Dev.	105.9054 (µm)
Mode size	186.3382 (µm)
Span	Off
Geo. mean size	141.9512 (µm)
Geo. variance	1.2467 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 56.1951 (µm) (9)90.00 (%)- 312.8649 (µm)
D10	56.19514 (µm)
D90	312.86487 (µm)
D (v0.1)	56.19514 (µm)
D (v0.5)	158.41113 (µm)
D (v0.9)	312.86469 (µm)
Replicate 2	02-07-2017
Median size	156.56572 (µm)
Mean size	163.81972 (µm)
Variance	7290.7 (µm2)

St. Dev.	85.3854 (µm)
Mode size	185.8120 (µm)
Span	Off
Geo. mean size	137.7019 (µm)
Geo. variance	1.2114 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 59.0109 (µm) (9)90.00 (%)- 274.2823 (µm)
D10	59.01091 (µm)
D90	274.28235 (µm)
D (v0.1)	59.01091 (µm)
D (v0.5)	156.56572 (µm)
D (v0.9)	274.28235 (µm)

Table 12. Particle Size Measurement – FBG 30 mins

Replicate 1	
Median size	626.54669 (µm)
Mean size	613.49268 (µm)
Variance	1.3097E+5 (µm2)
St. Dev.)	361.9040 (µm
Mode size	721.2449 (µm)
Span	off
Geo. mean size	421.5194 (µm)
Geo. variance	1.7634 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 48.3762 (µm)
D10	48.37621 (µm)
D90	1044.56958 (µm)
D(v0.1)	48.37621 (µm)
D(v0.5)	626.54669 (µm)
D(v0.9)	1044.56958 (µm)
Replicate 2	
Median size	623.10486 (µm)
Mean size	604.40875 (µm)
Variance	1.2642E+5 (µm2)
St. Dev.	355.5606 (µm)
Mode size	722.8904 (µm)

Span	Off
Geo. mean size	414.7040 (µm)
Geo. variance	1.7668 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 47.8762 (µm)
D10	47.87625 (µm)
D90	1017.00262 (µm)
D (v0.1)	47.87625 (µm)
D (v 0.5)	623.10486 (µm)
D (v 0.9)	1017.00262 (µm)

Table 13. Particle Size Measurement – FBG 40 mins

Replicate 1	
Median size	513.58270 (µm)
Mean size	501.90750 (µm)
Variance	1.0079E+5 (µm2)
St. Dev.	317.4706 (µm)
Mode size	630.8520 (µm)
Span	Off
Geo. mean size	334.2144 (µm)
Geo. variance	1.8071 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 42.8538 (µm) (9)90.00 (%)- 888.1856 (µm)
D10	42.85380 (µm)
D90	888.18561 (µm)
Replicate 2	
Median size	506.64450 (µm)
Mean size	494.65234 (µm)
Variance	1.0221E+5 (µm2)
St. Dev.	319.6994 (µm)
Mode size	630.9888 (µm)
Span	Off
Geo. mean size	324.7670 (µm)

Geo. variance	1.8338 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 41.2130 (µm) (9)90.00 (%)- 885.2012 (µm)
D10	41.21298 (µm)
D90	885.20117 (µm)

Table 14. Particle Size Measurement – FBG 45 mins

Replicate 1	
Median size	634.70795 (µm)
Mean size	629.80042 (µm)
Variance	1.0285E+5 (µm2)
St. Dev.	320.6972 (µm)
Mode size	716.8334 (µm)
Span	Off
Geo. mean size	481.2655 (µm)
Geo. variance	1.5086 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 104.8457 (µm) (9)90.00 (%)-1000.5261 (µm)
D10	104.84566 (µm)
D90	1000.52606 (µm)
Replicate 2	
Median size	621.47534 (µm)
Mean size	627.38251 (µm)
Variance	1.0996E+5 (µm2)
St. Dev.	331.5950 (µm)
Mode size	718.0587 (µm)
Span	Off
Geo. mean size	480.9795 (µm)
Geo. variance	1.4838 (µm2)

Diameter on cumulative %	(2)10.00 (%)- 146.3348 (µm) (9)90.00 (%)-1022.7184 (µm)
D10	146.33479 (µm)
D90	1022.71844 (µm)

Table 15. Particle Size Measurement – Agglomeration 90 mins

Replicate 1	02-07-2017
Median size	158.41113 (µm)
Mean size	175.00046 (µm)
Variance	11216 (µm2)
St. Dev.	105.9054 (µm)
Mode size	186.3382 (µm)
Span	Off
Geo. mean size	141.9512 (µm)
Geo. variance	1.2467 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 56.1951 (µm) (9)90.00 (%)- 312.8649 (µm)
D10	56.19514 (µm)
D90	312.86487 (µm)
D (v0.1)	56.19514 (µm)
D (v0.5)	158.41113 (µm)
D (v0.9)	312.86469 (µm)
Replicate 2	02-07-2017
Median size	156.56572 (µm)
Mean size	163.81972 (µm)

Variance	7290.7 (µm2)
St. Dev.	85.3854 (µm)
Mode size	185.8120 (µm)
Span	Off
Geo. mean size	137.7019 (µm)
Geo. variance	1.2114 (µm2)
Diameter on cumulative %	(2)10.00 (%)- 59.0109 (µm) (9)90.00 (%)- 274.2823 (µm)
D10	59.01091 (µm)
D90	274.28235 (µm)
D (v0.1)	59.01091 (µm)
D (v0.5)	156.56572 (µm)
D (v0.9)	274.28235 (µm)

Table 16. Limonene oxide content (g/g of sample) throughout the storage

Limonene oxide content (100000*LO (g/g of powder))					
Process	Week 0	Week 1	Week 2	Week 3	Week 4
Spray dried Batch	21.6	47.4	55.4	81.9	177.2
Agg run 30 mins	20	43	80	88	136
Agg run 60 mins	19	50.8	79.4	86	143.1
Agg run 90 mins	22	53	70.1	99	111.1
Gran 25 mins batch	11.2	26.2	48.8	48.8	41.8
Gran 35 mins batch	11.2	25.9	48.2	48.2	56.7
Gran 40 mins batch	33.5	40.9	61.2	61.2	80.7
Gran 45 mins batch	11.7	28.7	46.7	46.7	54

Table 17. LO/L ratio comparison

		1000*LO/L			
Process	Week 0	Week 1	Week 2	Week 3	Week 4
Spray dried Batch	0.85	2.3	2.587	5.907	12.015
Agg run 30 mins	0.73	2.3	4.688	5.23	7.792
Agg run 60 mins	0.585	2.55	4.753	5.659	8.844
Agg run 90 mins	0.885	2.95	3.83	6.11	6.74
Gran 25 mins	0.983	1.001	1.48	2.53	2.32
Gran 35 mins	0.94	1.001	1.29	2.51	2.623
Gran 40 mins	1.87	2.3	2.7	3.84	0.545
Gran 45 mins	1.04	1.056	1.53	2.25	2.643

Table 18. Operating parameters for granulation batch 1 – Granulation time of 25 mins

Weight of emulsion agglomerated – 512 g

START			**Temps**	**Temp**	**Exhaust air flap**	**Flow rate**	**weight of emulsion**
Time	**Function**	**Inlet**	**exhaust**	**Product**	**Damper**	**of emulsion**	**(g)**
00:00	Spray	80	60	53	0	25	1563
07:47	Spray	80	56	49	0	25	1366
10:00	Spray	80	56	49	0	25	n/a
15:00	Spray	80	56	49	0	25	n/a
21:00	Spray	80	59	55	0	25	1051
24:29	shut down					Weight of emulsion Agglomerated	512 g

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Table 19. Operating parameters for granulation batch 2 – Granulation time of 35 mins

Weight of Emulsion agglomerated – 1000 g

START			Temps		Exhaust air flap	Flow rate of emulsion	weight of emulsion
Time	Function	Inlet	exhaust	Product	Damper		(g)
00:00	Temp Adjusting	69	52	34	0	0	
02:17	Spray	81	61	55	0	25	1442
06:27	Spray	80	55	48	0	25	1328
10:43	Spray	80	55	48	0	25	1191
12:45	Spray	80	55	49	0	30	1116
15:45	Spray	80	54	48	0	30	1047
21:00	Spray	80	54	49	0	30	859
34:48	Shut Down	80	53	48	0	30	442

Table 20. Operating parameters for granulation batch 3 – Granulation time of 40 mins

Weight of emulsion agglomerated – 1941 g

START			**Temps**		**Exhaust air flap**	**Flow rate of emulsion**	**weight of emulsion**
Time	**Function**	**Inlet**	**exhaust**	**Product**	**Damper**		**(g)**
00:00	Spray	100	67	56	0	15	1942

05:00	Spray	100	67	57	0	15	1660
10:00	Spray	100	68	58	0	20	1556
15:00	Spray	100	67	57	0	20	1460
20:00	Spray	100	66	57	10	25	1335
25	Spray	100	65	57	10	25	1211
30	Spray	100	65	57	10	25	1068
35	Spray	100	63	57	10	25	910
40	Spray	100	64	57	10	25	797

Table 21. Operating parameters for Granulation batch 4 – Granulation time of 45 mins

Weight of emulsion agglomerated – 1456 g

START		Temp	Temps	Temp	Exhaust air flap	Flow rate	weight of emulsion
Time	Function	Inlet	exhaust	Product	Damper	of emulsion	(g)
08:45	Spray	80	56	50	0	25	1241
10:00	Spray	80	56	50	0	25	1147
15:00	Spray	80	56	51	0	25	1005
20:00	Spray	80	56	51	0	25	920
25:00	Spray	80	56	51	0	30	773
30:00	Spray	80	55	50	0	30	620
35:00	Spray	80	54	50	0	30	453
40:00	Spray	80	54	50	0	30	288
43:00	Closed spraying	80	55	52	0	30	0

46:00	shut down	74	54	37	0	0	0

Table 22. Moisture content of encapsulated samples after water activity equilibration.

Process	% Moisture content
Spray Drying	5.1
Agglomeration run 30 mins	8.8
Agglomeration run 60 mins	8.67
Agglomeration run 90 mins	8.52
Granulation run 25 mins	5.38
Granulation run 35 mins	4.15
Granulation run 40 mins	4
Granulation run 45 mins	3.1